Taxonomic and Molecular Studies of Bee Pollinators (Hymenoptera: Apoidea) from Kashmir Valley (J&K), India

PhD Synopsis

Scholar	**Irfan Ahmed Noorani**
Enrolment Number	**20162CUKmr004**
Date of Registration	**10 April 2021**
Supervisor	**Prof. Mohd Yousuf** **Professor** Department of Zoology, Central University of Kashmir
Co-Supervisor	**Dr. Sajad Hussain Parey** Assistant Professor Department of Zoology BGSB University Rajouri, J&K
Department	**Zoology**
School	**Life Sciences**

Central University of Kashmir
Ganderbal, J&K

Title: **Taxonomic and Molecular Studies of Bee Pollinators (Hymenoptera: Apoidea) from Kashmir Valley (J&K), India.**

Background

Jammu and Kashmir is a hilly state having varied topography and the agriculture forms the backbone of the state economy with over 70% of its population depending upon it for livelihood and employability. Presently agriculture sector is contributing 13.7% of the GDP at national level and at state level it is contributing around 21.09% (Kaloo and Choure 2015). The diversity of insect pollinator community significantly affects the pollination of important agricultural crops (Albrecht *et al.*, 2012). Insect pollination is one of the most important natural activities for the maintenance and promotion of biodiversity on the earth and many ecosystems including agro-ecosystem depend upon pollinators diversity to maintain overall biological diversity and its benefit to the society by increasing food security and improving the livelihoods (Khan and Khan 2004). Lack of sufficient number of suitable pollinators causes decline in fruit and seed production (Partap, 2001). The loss of pollinators is currently a matter of concern for ecologist and conservation biologists especially because of its clear relationship with consequent socioeconomic damage in both agricultural and natural areas (Burkle *et al.*, 2013). The different types of insect pollinators like bees, wasps, ants, flies, butterflies, moths, beetles and some vertebrates like some birds and mammals are the efficient and potential pollinators of different crops around the world. Out of the total 2,50,000 species of plants, 88.7% are flowering plants (Angiosperms) and about 90% of them are pollinated by insects. Of the total insect pollination activities, about 73% is performed by bees (Hoshiba and Sasaki, 2008). In the recent past, a worldwide decline has been observed in the number of bees. Various reasons have been recorded responsible in the decline of bees such as quality food shortage, human activities including habitat fragmentation and degradation, climate change and use of pesticides in agriculture (Potts *et al*., 2010).

Bees are belonging to a fascinating group of insects which have a diverse distribution worldwide and are considered most effective pollinators (Raj *et al.*, 2012). It is well proven fact that bees are essential in a terrestrial ecosystem consisting of wild and cultivated field crops, thus directly affecting the sustainability of the system and essential for the existence of other species, including human beings (Southwick and Southwick, 1992). Bees belonging to superfamily Apoidea of the order Hymenoptera of the class Insecta are known to have

evolved from the flower foraging wasps, which off lately left their age-old carnivorous habit as seen in aculeate wasps (Michener, 2007). The superfamily Apoidea has been further hierarchically classified into seven families, namely Apidae, Megachilidae, Halictidae, Andrenidae, Colletidae, Melittidae, and Stenotritidae of which family Strenotritidae is restricted to Australian region only while other six are widely distributed throughout world including India. Worldwide, around 25,000 bee species have been described till date and in India, 726 bee species belonging to 84 genera of 6 families have been recorded (Michener, 2007; Pannure, 2016; Ascher and Pickering, 2020).

In the Union Territory of Jammu and Kashmir, a consolidated checklist of bee species (Apoidea) is not available but different workers have reported large number of bee species from both Jammu region as well as Kashmir region independently. In one study, Ara *et al.* (2019) have reported 37 bee species from Kashmir region belonging to 15 genera. Similarly, Abrol *et al.* (2012) studied the diversity of non-Apis bee species from six districts (Kathua, Samba, Jammu, Udhampur, Ramban and Doda) of Jammu region and reported a total of 30 morpho-species. In addition, Kumari and Sidhu (2020) identified 13 species belonging to 6 genera from four districts (Reasi, Udhampur, Kathua and Samba) of Jammu region.

So there is great scope of work to trace out the Taxonomy, Diversity and Molecular studies of Bees in Jammu and Kashmir, hence the current research problem is taken to fill this gap on Apoidea of Kashmir valley.

Review of Literature

Hussain *et al.* (2012) explored the diversity of hymenopteran pollinators at different altitudes of Himalayan foot hills of Pakistan. A total of 9 species were collected from diverse agro-ecosystems comprising of the orchards of pome and stone fruits. The lowest diversity of hymenopteran pollinators was found in monoculture fruits habitats whereas the diversity of pollinators was greater in multiple culture habitats.

Thakur and Mattu (2014) surveyed the diversity and distribution of insect visitors on different horticulture crops in Himachal Pradesh. The studies on different temperate crops viz., apple, pears, cherry, peach, plum and almond in Shimla hills revealed 70 species of insect visitors belonging to 06 orders and 27 families of class insecta. Out of 70 species, 24 belong to the order hymenoptera, 24 to Diptera, 16 to Lepidoptera, 03 to Coleoptera and 01 to Thysanoptera. The 24 species of order Hymenoptera belong to 11 families of which 08

species were from the family vespidae, 03 each from bombidae and apidae, 02 each from halictidae and formicidae and 01 each from Andrenidae, Xylocopidae, Ceretinidae, Tenthridinidae, Ichneumonidae and Scoliidae.

Paray *et al.* (2014) explored the diversity of pollinators from agro ecosystems of Kashmir Himalayas, comprising the apple orchards at different altitudes. They conducted the field experiments of 51 commercial fruit orchards in three main apple producing districts viz. Baramulla, Pulwama and Shopian. They have collected 970 specimens belonging to the main insect orders Hymenoptera and Diptera. The lowest diversity among hymenopterans was found in district Baramulla while the highest diversity was found in the orchards of district Shopian. In Dipterans the lowest diversity was found in the district Baramulla and highest in district Shopian. A total of 15 species belonging to 8 genera of 4 families (Xylocopidae, Apidae, Andrenidae and Halictidae) were reported from order hymenoptera. Among the wild bees the *Lasioglossum nursei* (Halictidae) was most diversified species in all the three districts and *Andrena patella* (Andrenidae) was least diversified species in all the three districts. The abundance, richness and evenness of the hymenopterous pollinator bees were found at peak during the flowering period of apple in the months of April to May.

Akhter *et al*. (2016) have carried out a comparative study on pollination potential of various hymenopteran insects of some crops having agricultural, horticultural and medicinal importance in Kashmir. The contribution of pollination potential of honey bees, bumble bees, carpenter bees, mining bees and wasps was analysed on the basis of their abundance and pollen load, carried on the their body. They found that among the selected crops almost 80% were mainly pollinated by honey bees. The *Apis spp*. because of their abundant population, special morphological characters like hairy body and pollen basket, diverse food preference constitute the most efficient and major group of crop pollinators.

Makkar *et al.* (2016) recorded mining bee *Andrena agilissima*, for the first time in India from the western agro-climatic zone of Punjab state and presented the first account of morphological and molecular characteristics of *A. agilissima*. The newly recorded species has increased the number of mining bee species to 21 in India. They studied taxonomic and metric values of total 40 morphological characters. Using the standard barcoding protocols, cytochrome c oxidase subunit 1 marker (standard DNA barcode region) based 658 bp DNA barcode sequence of the species was established, which marked the first step towards the DNA barcode library of solitary bees of Punjab. The barcode sequence generated for the

species were registered by GenBank, National Centre for Biotechnology Information (NCBI) and Barcode of Life Data (BOLD) Systems.

Dar *et al*. (2018) studied the insect pollinators of peach *Prunus persica* in the temperate conditions of Kashmir valley. They reported total 46 pollinator species in the landscapes of Budgam belonging to two orders Hymenoptera and Diptera. The most dominant hymenopteran flowers visitor families were Halictidae (45.84%), Andrenidae (11.64%), Apidae (5.76%) and Megachilidae (4.25%). Of all the bee species genus *Lasoglossum* and *Andrena* were most abundant visitor of the flowers of *Prunus persica.*

Saini *et al.* (2018) studied the diversity of bees (Hymenoptera; Apoidea) in and around Namdapha National Park, Arunachal Pradesh. They reported a total of 49 speceis under 12 genera belonging to 03 families viz. Apidae, Halictidae and Megachilidae. Family Megachilidae was recorded for the first time from Arunachal Pradesh.

Murao and Gibbs (2019) recorded new species *Halictus hedini hedini* of sweat bee based on DNA barcoding and morphology from Japan. The species had been previously mistaken for the Holarctic species *H. rubicundus*. *Halictus hedini hedini* is similar to *H. rubicundus*, but was separated by the first metasomal tergum with coarse and deep punctures in both sexes and the male flagellum ventrally with a fringe of short hairs.

Kaundil and Thakur (2020) conducted a preliminary study on insect pollinators in apple crop in different geographical zones of Himachal Pradesh. A total of 34 insects belonging to 05 orders and 11 families of class insecta were recorded. Out of 34 species, 13 species were from hymenoptera, 12 from diptera, 05 from Lepidoptera, 03 from Coleoptera and 01 from order Thysanoptera. Hymenopteran visitors recorded are belonging to 04 families namely Apidae, Halictidae, Vespidae and Ichneumonidae.

Hooda and Jain (2020) conducted a detailed survey on the diversity of bees (Hymenoptera: Apoidea) in Kota (Rajasthan) and reported 44 species. These bees of South east Rajasthan belong to 19 genera of 04 families viz. Apidae, Megachilidae, Halictidae and Andrenidae. Out of the four families Apidae was most diverse with 17 species and second being Megachilidae having 16 species.

Darbey *et al*. (2020) revealed similar patterns in native bee communities across public and private grasslands of eastern North Dokota, USA by molecular sequencing and morphological identification. They found the 28S LSU locus to be more capable of

discriminating between species than the 18S SSU rRNA locus and in some cases even resolved instances of cryptic species or morphologically ambiguous species complexes.

Sreebha and Femi (2020) documented the diversity of insect pollinators visiting the *Boerhavia diffusa* in Virudhunagar, Tamil Nadu. They reported 14 species of insect pollinators belonging to 04 orders: Lepidoptera, Hymenoptera, Diptera and Coleoptera and 09 families of these orders. The high and equal percentage of species diversity visiting *Boerhavia diffusa* were recorded form Hymenoptera and Lepidoptera.

Mir *et al*. (2020) conducted a study on Hymenopteran insect pollinators, visiting the crops of agriculture and horticulture in Baramulla district of Jammu and Kashmir. They reported a total of 12 insect species belonging to five families i.e., Apidae, Vaspidae, Formicidae, Halictidae and Xylocopidae of order hymenoptera.

Pakrashi *et al.* (2020) generated 156 DNA barcodes, of which 141 belonged to 30 morphologically identified bees from the Indian Himalayan Regions. The generated barcode data along with 84 sequences of global database distinctly helped in discriminating all the studied species with sufficient genetic distances and cohesive monophyletic clustering in Bayesian analysis (BA) phylogeny. The species delimitation methods, Automatic Barcode Gap Discovery (ABGD), Bayesian Poisson-Tree-Processes (bPTP), and General Mixed Yule-coalescent (GMYC) yielded 68, 70, and 71 molecular operational taxonomic units (MOTUs) respectively. On the basis of DNA barcode examination they detected the possible cryptic diversity in two *Apis* species (*A. cerana* and *A. dorsata*), *Bombus hypnorum, Lepidotrigona arcifera,* and *Ceratina sutepensis*. This novel work also evidenced the species complexes within *Bombus albopleuralis* and *Bombus trifasciatus* in this particular region. The species delimitation methods also helped in detecting an additional seven putative species from the Indian Himalayan Regions.

Hossain *et al.* (2021) conducted a study on the diversity and abundance of pollinators in different winter crops at Sher-e-Bangla Agricultural University Campus Dhaka. They identified a total of 15 species from winter crops viz., mustard, coriander, niger, black cumin and linseed. The species diversity reported was high in mustard while it was low in linseed. Honey bees and sweat bees were more abundant pollinators of the winter crops.

Research Gap

Since, scientific literature is very scanty on the diversity of bees of Kashmir and their molecular taxonomy remains largely unexplored from this particular region of India. Therefore, keeping in view the importance of bees as pollinators, their benefits to the society by increasing food security and improving livelihood the present study shall be undertaken to provide a comprehensive data regarding the diversity of bees of Kashmir Valley. Further DNA barcoding studies shall help in the species identification and establishing the phylogenetic relationship between different taxa. The proposed work shall not only provide a strong impetus for further research in future but also help in the conservation of the pollinators.

Objectives

- To collect and identify Bees (Apoidea) from different areas of Kashmir Valley.
- To enlist various Host plants foraged by different Bee species of Apoidea for studying plant-pollinator network.
- To perform DNA barcoding for confirming the morphological identification of the species and establishing the phylogenetic relationships.
- To describe new taxa (if any) based on morphological and molecular studies.

Hypothesis

Since much work has not been carried out on Bees in Kashmir valley of India, so in the present study the following research work shall be hypothesized;

1. Morpho-taxonomy will help in the identification of different species of Bees and preparation of checklists.
2. The sequencing of short fragment of mitochondrial cytochrome c oxidase subunit 1 (Cox1) gene from collected specimens and comparison with library of DNA barcode will further confirm the identification of different bee species and unravelling of any new species**.** Further the accurate phylogenetic information and evolutionary divergence of different Bee species can be elucidated through molecular taxonomy.
3. The combined approach of morphological taxonomy and molecular taxonomy in present study may help in illustrating the degree of relationship among the families and sub-families of Apoidea and also with other Super families of order hymenoptera.

Proposed Methodology

Collection

Specimens shall be collected from diverse sites of Kashmir region (J&K), India during the months of April to September by conducting the vigorous surveys over different areas with the help of sweep nets or by hand picking. The collection shall be made through random sampling method in the fields of agriculture and horticulture, gardens, grasslands roadsides and other open places where nectiferous flowers are abundant.

Preservation

The collected specimens shall be preserved for studying the morphological features and molecular analysis. Some collected specimens shall be preserved dry in order to retain their natural colour, other morphological characters and rest of the specimens shall be preserved in 70% alcohol for studying their different morphological structures under microscope. For molecular studies collected bees shall be preserved in 95% alcohol at -20^0 C in deep freezer.

Identification

The morphological identification of specimens shall be performed with the help of Stereozoom Binocular /Trinocular microscope and photography. For identification, the morphological structures like antennae, legs and other appendages shall be dissected out and permanent slides shall be prepared and studied. Dichotomous keys proposed by different workers (Michener 2007; Saini *et al.,* 2018) shall be followed for family/genus and species level morphological identification of the specimens.

Plant-Pollinator Network

Data analysis on Pollinator-Plant network shall be performed by Bipartite package implemented in R version 4.1.0 (R Core team 2021). The data on plant-pollinators shall be obtained during the field studies by getting pollinator abundance, plant species observed and getting the coordinates of the study area.

Molecular Analysis

For studies on phylogenetic relationship within Apoidea, the DNA Barcoding method based on sequencing of the standardized segment of the mitochondrial cytochrome c oxidase

subunit 1 (Cox1) gene (Hebert *et al*., 2003) shall be followed. The DNA shall be extracted from the legs or thorax of the specimen preserved in 95% alcohol by following the procedures of Ivanova *et al*. (2006) using Qiagen DNeasy Blood Tissue Kits (QIAamp Micro Kit) or Nucleospin DNA insect Mini Kit. The extracted DNA shall be amplified by Polymerase Chain Reaction (PCR) by following sequencing protocols as described by Hebert *et al*. (2003). The full length Cox1 DNA barcodes (658 bp) shall be amplified with standard primers LCO1490 & HCO2198 (Folmer *et al.,* 1994) or newly designed primers BeeCox1F1 & BeeCoxR2 (Bleidorn and Henze, 2021). The sequence of the gene of interest shall be generated at the designated centres of barcoding. The barcode sequences generated for the species shall be submitted to GenBank, National Centre for Biotechnology Information (NCBI) and Barcode of Life Data (BOLD) Systems to compare with reference database for reliable taxonomic identification and establishing phylogenetic relationship within the Apoidea.

References

Abrol, D. P., Shankar, U., Chatterjee, D. and Ramamurthy, V. V. (2012). Exploratory studies on diversity of bees with special emphasis on non-Apis pollinators in some natural and agricultural plants of Jammu division, India. *Current Science*. **103**(7): 780-783.

Akhter, F., Khanday, A. L. and Ahmad, S. T. (2016). Pollination potential: A comparative study of various hymenopteran insects pollinating some economically important crops in Kashmir. *International Journal of Advanced Research in Biological Sciences.* **3**(9): 50-59.

Albrecht, M., Schmid, B., Hautier, Y. and Müller C. B. (2012). Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society British: Biological sciences*. **279**: 4845-52.

Ara, S., Rather, Z. A., Paray, M. A., Khursheed, R. and Yaqoob. M. (2019). Bee flora of Kashmir: The Himalayan biodiversity hotspot. *Journal of Pharmacognosy and Phytochemistry*. **8**(2): 2172-2181.

Ascher, J. S. and Pickering, J. (2020). Discover Life bee species and world checklist (Hymenoptera: Apoidea: Anthophila). http://www.discoverlife.org./mp/20q?guide = Apoidea_species.

Bleidorn, C. and Henze, K. (2021). A new primer pair for barcoding of bees (Hymenoptera: Anthophila) without amplifying the orthologous coxA gene of *Wolbachia* bacteria. *BMC Research Notes*. **14**(427): 1-6.

Burkle, L. A., Marlin, J. C. and Knight, T. M. (2013). Plant pollinator interactions over 120 years: loss of species, co-occurrence, and function. *Science*. **339**: 1611–1615.

Dar, S. A., Mir, G. M., Wani, A. R., Kandoo, A. A. and Nissar, M. (2018). Insect pollinators of peach *Prunus persica* in landscape of temperate India. *International Journal of Chemical Studies.* **6**(3): 1281-1288.

Darby, B., Bryant, R., Keller, A., Jochim, M., Moe, J., Schreiner, Z., Pratt, C., Euliss, N. H., Park, M., Simmons, R. and Otto, C. (2020). Molecular sequencing and morphological identification reveal similar pattern in native bee communities across public and private grasslands of eastern North Dokata. *PLoS ONE*. **15**(11): e0227918.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. C. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*. **3**(5): 294-299.

Hebert, P. D. N., Cywinska, A., Ball, S. L. and Dewaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of Royal Society of London Series B: Biological Sciences.* **270**: 313-321.

Hooda, S. and Jain, N. (2020). Diversity of Bees (Hymenoptera: Apoidea) in Kota, Rajasthan (India). *Journal of Environment and Bio-Sciences*. **34**(1): 65-68.

Hoshiba, H. and Sasaki, M. (2008). Perspective of multi-modal contribution of honeybee resources in our life. *Entomological Research*. **38**: S15-S21.

Hossain, M. S., Rahman, M. M., Hossain, M. E., Sarkar, S. and Rahman, K. (2021). Diversity and Abundance of Pollinators in Different Winter Crops at Sher-E-Bangla Agricultural University Campus, Dhaka. *Journal of Biodiversity Conservation and Bioresource Management* **7**(1): 33-42.

Hussain, A., Khan, M. R., Ghffar, A., Hayat, A. and Jamil, A. (2012). The hymenopterous pollinators of Himalayan foot hills of Pakistan (distributional diversity). *African Journal of Biotechnology*. **11**(28): 7263-7269.

Ivanova, N. V., Dewaard, J. R. and Hebert, P. D. N. (2006). An inexpensive automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* **6**(4): 998-1002.

Kaloo, M. J. and Choure, P. T. (2015). Present Status and Future Prospectus of Agriculture in Jammu and Kashmir. *IOSR Journal of Humanities and Social Science*. **20**(11): 62-67.

Kaundil, P. and Thakur, R. K. (2020). A Preliminary Study on Insect Pollinators in Apple Crop in Different Geographical Zones of Himachal Pradesh. *International Journal of Current Microbiology and Applied Sciences*. **9**(11): 325-331.

Khan, M. R. and Khan, M. R. (2004). The role of honey bees *Apis mellifera* L. (Hymenoptera: Apidae) in pollination of apple. *Pakistan Journal of Biological Science*. **7**: 359-362.

Kumari, P. and Sidhu, A. K. (2020). Diversity of apoidea (Hymenoptera) from Grassland ecosystem of Jammu (UT of Jammu and Kashmir). *Journal of Entomology and Zoology Studies*. **8**(4): 424-427

Makkar, G. S., Dey, D. and Chhuneja, P. K. (2016). Mining bee *Andrena (Agandrena) agilissima* (Hymenoptera: Andrenidae): A new record from India with morphological and molecular notes. *Journal of Applied and Natural Science* **8**(4): 1775-1778.

Michener, C. D. (2007). The Bees of the World. *The John Hopkins University Press, second edition, Baltimore, MD London.* 952 pp.

Mir, S. J., Jamali, M. M. and Majeed, M. (2020). Studies of hymenopteran insect pollinators in district Baramulla of Jammu and Kashmir. *International Journal of Agricultural & Biosciences* **1**(1): 1-5.

Murao, R., and Gibbs, J. (2019). *Halictus hedini hedini* (Hymenoptera: Halictidae) newly recorded from Japan, revealed by DNA Barcoding and morphology. *Journal of Melittology* **84**: 1-8.

Pakrashi, A., Kundu, S., Saini, J., Kaomud Tyagi, K., Chandra, K. and Kumar, V. (2020). Molecular identification of selected bees from the Indian Himalaya: A preliminary effort. *Journal of Asia-Pacific Entomology*. **23**: 883-889.

Pannure, A. (2016). Bee pollinators decline: perspectives from India. *International Research Journal of Natural and Applied Sciences*. **3**(5): 1-10.

Paray, M. A., Parey, S. H., Yaqoob, M., Khursheed, R., Bhat, B. H., Gupta, S. and Rather, Z. A. (2014). The pollinators of apple orchards of Kashmir valley (India) (distributional diversity). *Ecology, Environment and Conservation* **20**: 471-477.

Partap, T. (2001). Mountain agriculture, marginal land and sustainable livelihoods: Challenges and opportunities. *International Symposium on Mountain Agriculture in HKH region. ICIMOD, Nepal, (21-24 May 2001)*.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. (2010) Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology & Evolution.* **25**(6): 345–353.

Raj, H., Mattu, V. K. and Thakur, M. L. (2012). Pollinator diversity and relative abundance of insect visitors on apple crops in Shimla hills of western Himalaya, India. *International Journal of Science and Nature*. **3**(3): 507–513.

R. Core Team (2021). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org*

Saini, J., Chandra, K., Kumar, H., Ghosh, D., Kazmi, S. I., Payra, A. and Deepak, C. K. (2018). Diversity of Bees (Hymenoptera: Apoidea) in and around Namdapha National Park, with an Updated Checklist from Arunachal Pradesh, India. *Rec. Zoological Survey of India*. **118**(4): 413-425.

Southwick, E. E. and Southwick, L. (1992). Estimating the economic value of honey bees (Hymenoptera: Apidae), as agricultural pollinators in the United States. *Journal of Economic Entomology*. **85**(3): 621–633.

Sreebha, R., and Femi, G. D. (2020). Diversity of insects pollinators visiting *Boerhavia diffusa* L.(Nyctaginaceae) a common weed in Virudhunagar Block, Tamil Nadu. *International Journal of Botany and Research*. **10**(2): 15-22.

Thakur, B., and Mattu, V. K. (2014). Diversity and distribution of pollinators of temperature fruit crops of Shimla Hills in Himachal Pradesh. *Asian Journal of Advanced Basic Sciences*. **3**(1): 151-163.